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## **P-TEFb: finding its ways to release promoter-proximally paused RNA Polymerase II**

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### **Abstract**

The release of a paused Pol II depends on the recruitment of P-TEFb. Recent studies showed that both active P-TEFb and inactive P-TEFb (7SK snRNP) can be recruited to the promoter regions of global genes by different mechanisms. Here, we summarize the recent advances on these distinct recruitment mechanisms.

### **Key words**

P-TEFb, 7SK snRNP, Brd4, SEC, KAP1, RNA Pol II, promoter-proximal pausing, transcription elongation

## Introduction

The eukaryotic transcription by RNA polymerase II (Pol II) involves several tightly coordinated steps. While the regulation of transcription initiation has been a long-held paradigm, the transcription elongation is recently regarded as another critical step for governing the expression of inducible genes.<sup>1-3</sup> As early as 1992, the *in vitro* transcription assay revealed the existence of both positive and negative transcription elongation factors.<sup>4</sup> The factor with the capacity to promote transcription elongation was later characterized as P-TEFb (positive transcription elongation factor b), a heterodimer kinase composed of CDK9 and its cyclin partner (primarily Cyclin T1 and T2).<sup>5</sup> The factors with negative role in elongation were identified as DSIF (DRB sensitivity-inducing factor, composed of Spt4 and Spt5) and NELF (negative elongation factor, composed of NELF-A, B, C/D, E subunits).<sup>6, 7</sup> Shortly after transcription initiation, DSIF and NELF bind to the initiated Pol II at the promoter-proximal region, 20-60 nucleotides downstream of transcription start site, inducing the promoter-proximal pausing of Pol II.<sup>8, 9</sup> Of importance, growing evidence has indicated that this pausing is a checkpoint for governing the quick expression of 30-70% of the transcriptionally active genes in metazoans.<sup>2, 3, 10</sup> The release of paused Pol II depends on the recruitment of P-TEFb to promoter regions, where P-TEFb phosphorylates DSIF, NELF and the Ser2 of Pol II C-terminal domain (CTD). These P-TEFb-mediated phosphorylation events lead to the eviction of NELF from paused Pol II and the transition of Pol II from pausing into productive elongation, thereby allowing cells to rapidly and efficiently cope with various challenges.<sup>1, 3, 8, 11</sup>

The molecular mechanism that regulates the activity and recruitment of P-TEFb has been a field of interest to many researchers in the beginning of this century. Fifteen years' efforts on this field have drawn out a sketch depicting how the activity and recruitment of P-TEFb is tightly regulated. In cells, the majority of P-TEFb is sequestered in an inactive 7SK snRNP complex that contains 7SK snRNA and three nuclear proteins HEXIM1 (Hexamethylene Bisacetamide Inducible 1), MePCE (Methylphosphate Capping Enzyme)

and LARP7 (La Ribonucleoprotein Domain Family Member 7).<sup>11-13</sup> Within this complex, MePCE and LARP7 bind to 7SK and stabilize 7SK RNA, whereas 7SK serves as a scaffold to mediate the interaction between HEXIM1 and P-TEFb, thereby maintaining P-TEFb in an inactive state.<sup>11-13</sup> Upon stress, the core P-TEFb is released from 7SK snRNP and is recruited to the target promoters via the P-TEFb recruitment factors.<sup>11, 12, 14, 15</sup> Ample evidence indicates that the bromodomain containing protein Brd4 and the super elongation complex SEC are capable of recruiting active P-TEFb to promoters.<sup>11, 16-18</sup> Brd4 belongs to BET family that contains two bromodomains and an extraterminal domain. Upon stimulation, Brd4 binds to and recruits active P-TEFb to promoter regions to release paused Pol II.<sup>14, 16, 19-21</sup> SEC is a multisubunit complex consisting of four AFF scaffold proteins (AFF1 to AFF4), one of three ELL proteins (ELL1 to ELL3), and an ENL (or its analogue AF9).<sup>18</sup> Similar to Brd4, SEC is able to interact with and recruit active P-TEFb to promoters for the release of paused Pol II.<sup>11, 16-18</sup>

Of interest, while the active P-TEFb has been proposed to be the major form that is inducibly recruited to gene promoters upon stimulation, several recent studies showed that the inactive P-TEFb in the 7SK snRNA complex can also be loaded onto a vast array of promoters for the release of paused Pol II either in basal or stimulation state.<sup>22-24</sup> Although the recruitment of inactive 7SK snRNP and the release of P-TEFb at promoter employ very diverse mechanisms, nevertheless, it represents another approach to release the promoter-proximally paused Pol II. To distinguish these two distinct recruitment mechanisms, we refer to the recruitment of active form of P-TEFb as the canonical P-TEFb recruitment model and the recruitment of inactive P-TEFb in complex with 7SK snRNP as the non-canonical P-TEFb recruitment model. In this short review, we summarize recent advances in dissecting these two kinds of recruitment models.

### **The canonical P-TEFb recruitment model**

Ample evidence indicates that in metazoans, the promoter-proximal pausing of Pol II serves as a key step for controlling the expression of inducible genes that are critical for cell proliferation, differentiation, and environment response. Although this pausing allows

fast response in gene expression,<sup>3</sup> the danger, however, is that mistakes in releasing the paused Pol II may doom the fate of the cell beyond return. Hence, the release of paused Pol II must be tightly controlled. To achieve this, the release of P-TEFb from 7SK snRNP, the availability of Brd4 and SEC, and the recruitment of P-TEFb must be coordinated and strictly regulated.

For P-TEFb release, as revealed in our previous studies,<sup>15, 21</sup> stress (such as UV, DOX, and HMBA), induces the activation of protein phosphatase PP2B and PP1 $\alpha$  signal pathways and these two pathways work together to open the “lock” that detains P-TEFb in the inactive 7SK snRNP.<sup>12, 13</sup> In this process, stress-activated PP2B acts first to induce the conformational change of nucleoplasmic 7SK snRNP complex to expose the concealed T-loop of Cdk9. This enables PP1 $\alpha$  to access and dephosphorylate the T186ph of Cdk9 T-loop, leading to the release of P-TEFb from 7SK snRNP (Fig. 1A).<sup>15</sup> Meanwhile, to release the chromatin-bound Brd4, stress-activated histone deacetylases HDAC1/2/3 work together with PP1 $\alpha$  pathway to open another “lock” that sequesters almost all of Brd4 on chromatin in unstimulated state.<sup>20, 21</sup> In this case, PP1 $\alpha$  acts first to dephosphorylate the phospho-Ser10 of nucleosomal histone H3 (H3S10ph). This allows HDACs to access to and deacetylate the acetylated K5 and K8 of nucleosomal H4 (H4K5ac/K8ac), thereby releasing the chromatin-bound Brd4 to recruit the active P-TEFb.<sup>20, 21</sup> Moreover, with a shared PP1 $\alpha$  signal pathway, stress can induce the release of P-TEFb from 7SK snRNP and the release of chromatin-bound Brd4 at the same time and coordinate the recruitment of active P-TEFb simultaneously (Fig. 1A).<sup>15, 21</sup>

Moreover, even for the pause release, our most recent study revealed that Brd4 and SEC also work together to release a promoter-proximally paused Pol II by recruiting multiple P-TEFbs (Fig. 1B).<sup>16</sup> In this process, the Mediator subunits (Med1 and Med23) and the transcription factor Tat-SF1 constitute a Brd4-P-TEFb complex-specific recruitment pathway. Upon stress, Brd4 delivers the first P-TEFb to DSIF via the recruitment pathway, leading to the phosphorylation of Spt5 subunit of DSIF. Meanwhile, AFF1-SEC/AFF4-SEC recruits the second P-TEFb to NELF-E via Med26, and

AFF1-ENL-SEC/AFF1-AF9-SEC recruits the third P-TEFb to NELF-A via Paf1c, leading to the phosphorylation of NELF-E and -A. These three phosphorylation events result in the eviction of NELF from paused Pol II and the release of Pol II into gene body. Then, AFF4-ENL-SEC/AFF4-AF9-SEC brings the forth P-TEFb to Paf1c to phosphorylate Pol II CTD at Ser2, which facilitates the 3'-end processing of mRNA. The pause release is regulated by the cooperation of multiple P-TEFbs which are recruited by Brd4 and SEC subtypes via a Mediator- and Paf1c-coordinated recruitment network (Fig. 1B).<sup>16</sup>

Therefore, the canonical P-TEFb recruitment model at least involves the following steps: the release of P-TEFb from 7SK snRNP, the release of chromatin-bound Brd4 and the recruitment of multiple P-TEFbs by Brd4 and SEC. Each step is tightly controlled by at least two signal pathways so that to prevent the inappropriate release of the paused Pol II (Fig.1). Of note, our previous data implicated that most of SEC's components might associate with chromatin in unstimulated cells, and may need to be released as free form upon stress,<sup>16</sup> suggesting the existence of a yet-to-be-identified step of signal-induced release of chromatin-associated SEC components.

### **The non-canonical P-TEFb recruitment model**

Comparing to the canonical model, the non-canonical P-TEFb recruitment model involves the recruitment of 7SK snRNP and the release of P-TEFb at promoter ("on site") (Fig. 2). The association of 7SK snRNP with HIV promoters was firstly reported in 2010 by D'Orso, et. al.<sup>25</sup> Most recently, D'Orso's Lab demonstrated that transcriptional regulator KAP1 (also known as Trim28 and TF1b) was able to interact with LARP7 and tether 7SK snRNP to the promoters of primary response genes (PRGs) in respond to stimuli.<sup>23, 24</sup> Genome-wide studies showed that KAP1 and 7SK snRNP co-localized on most promoters containing paused Pol II.<sup>23</sup> Depletion of KAP1 reduced the promoter occupancy of 7SK snRNP and impeded the transcription elongation.<sup>24</sup> However, how P-TEFb is released from 7SK snRNP "on-site" in this case remains unknown.

Interestingly, in 2013, Fu's Lab found that SR-splicing factor SRSF2 (also known as

SC35) was able to interact with both 7SK RNA and promoter-associated nascent RNA and was recruited to promoter as a component of 7SK snRNP complex.<sup>26</sup> High throughput ChIP-Seq analysis showed that SRSF2 could be loaded on promoter regions of a vast array of genes. Through the interaction between SRSF2 and exonic-splicing enhancer (ESE) RNA, ESE RNA coordinated the release of SRSF2 and the release of P-TEFb from the 7SK snRNP at promoter, thereby leading to the transcription activation.<sup>26</sup>

Meanwhile, Rosenfeld's lab identified that the recruitment of Brd4-dependent demethylase JMJD6 on enhancer regions was crucial for regulating the pausing release of a subset of genes.<sup>27</sup> They found that JMJD6 bound to the C-terminus of Cdk9 and was co-recruited with 7SK snRNP to enhancers of ~1022 genes, where JMJD6 removed the cap structure of 7SK RNA to induce its degradation, reducing the stability of 7SK snRNP and promoting the "on site" release of P-TEFb.<sup>27</sup> Another example of "on-site" release of P-TEFb by remodeling 7SK RNA is DDX21.<sup>28</sup> DDX21 is a DEAD-box RNA helicase capable of unwinding RNA in ATP-dependent manner. It binds to 7SK RNA and, as a component of 7SK snRNP, is recruited to the promoters of genes encoding snoRNAs and ribosomal proteins.<sup>28</sup> Both *in vitro* and *in vivo* assays indicate that DDX21 can release P-TEFb from 7SK snRNP through remodeling the secondary structure of 7SK RNA, allowing the transcription elongation of target genes.<sup>28</sup>

In addition to remodeling 7SK RNA, PPM1G has been shown capable of releasing P-TEFb "on-site" by modifying T-loop of Cdk9.<sup>29</sup> PPM1G is a protein phosphatase and is recruited to promoters by transcription factor NF- $\kappa$ B and HIV-encoded Tat protein.<sup>29, 30</sup> By dephosphorylating T186p of Cdk9, PPM1G disrupts the 7SK snRNP "on site" to release P-TEFb to promoters.<sup>29</sup>

Besides protein factors, PSA eRNA (an androgen receptor-regulated enhancer RNA) has also been implicated in the disassembly of 7SK snRNP and the pause release of ~674 genes.<sup>31</sup> In this case, PSA eRNA forms a secondary structure which is highly similar to the 3'-end of 7SK RNA. PSA eRNA can then compete with 7SK for binding to Cyclin T1

subunit of P-TEFb, extracting P-TEFb out from 7SK snRNP "on-site".<sup>31</sup>

Taken together, the major feature of the non-canonical model is the loading of whole inactive 7SK snRNP complex, not active P-TEFb, onto the promoter or enhancer regions of target genes. Although the "on-site" release of P-TEFb from 7SK snRNP seems not as strict as in the canonical model, the diverse "on-site" P-TEFb release mechanisms, nevertheless, may lend cells the ability to accommodate the transcriptional needs in response to diverse challenges. Moreover, "on-site" activation enables 7SK snRNP to release P-TEFb in proximity to the paused Pol II, allowing the quick response to various stimulations.

### **Whether the two recruitment models can be reconciled with each other in cells?**

Given that both canonical and non-canonical mechanisms play roles in recruitment of P-TEFb in eukaryotic cells, it raises an intriguing question: whether the two mechanisms can be reconciled with each other in cells under basal condition or after stimulation. Of interest, Fu's Lab found that SR protein SRSF2 not only interacted with 7SK snRNP, but also associated with active P-TEFb recruitment factor Brd4.<sup>26</sup> They showed that RNAi depletion of SRSF2 could remarkably reduce the recruitment of P-TEFb, but did not affect the promoter occupancy of HEXIM1 and Brd4. While depletion of Brd4 significantly reduced the promoter recruitment of both SRSF2 and P-TEFb, it had no effect on the promoter enrichment of HEXIM1.<sup>26</sup> These data raise a possibility that while ESE RNA induces the "on-site" release of SRSF2 and P-TEFb from 7SK snRNP, SRSF2 interacts with P-TEFb and presents itself, together with P-TEFb, to Brd4. Then Brd4 recruits P-TEFb to Spt5 subunit of DSIF via the recruitment pathway consisting of Med1, Med23 and Tat-SF1. In this way, SRSF2 connects two recruitment mechanisms together to accommodate the transcription needs even under the basal state.

Another case is RelA subunit of NF- $\kappa$ B transcription factor. Brd4 has been shown to bind to NF- $\kappa$ B via interaction with acetylated-K310 of RelA in response to TNF $\alpha$  stimulation.<sup>19</sup> This binding facilitates the recruitment of P-TEFb to the promoters of



NF- $\kappa$ B target genes, including *IL-8*. On the other hand, 7SK snRNP is shown to associate with the *IL-8* promoter. Upon TNF $\alpha$  stimulation, RelA can recruit PPM1G to *IL-8* promoter, where PPM1G induces the release of P-TEFb from 7SK snRNP by dephosphorylating T-loop of Cdk9. Depletion of PPM1G results in the enrichment of HEXIM1 and LARP7 on *IL-8* promoter but the decrease of P-TEFb in *IL-8* gene body.<sup>29</sup>

<sup>30</sup> Combining these reported data, it is possible that the RelA-bound Brd4 might function as a "receptor" to accept the PPM1G-released P-TEFb and then recruits the P-TEFb to Spt5 of DSIF. Hence, RelA might play a role in coordinating the two recruitment mechanisms upon stimulation.

### Perspectives

Over the past decade, our understanding on transcription elongation has been continuously updated. While the active P-TEFb has been thought to be the major form being recruited to promoters of target genes, recent advances indicate that the inactive 7SK snRNP can be the another choice to be deposited to promoters. However, several points still remain to be addressed. One intriguing question is whether these two recruitment mechanisms are reconciled with each other in cells. Although the above mentioned examples imply this possibility, it apparently needs direct evidence. Another point is that although KAP1 has been shown capable of recruiting 7SK snRNP to promoters of global genes, how the promoter-associated 7SK snRNP selectively releases its P-TEFb at promoter or enhancer regions that belong to distinct signal pathways is still unclear. Similarly, for the canonical model, how the active P-TEFb is specifically delivered to the target promoters in response to a given stimulation is murky. Finally, it has been widely accepted that the signal-dependent dephosphorylation at T186ph of Cdk9 T-loop by protein phosphatase, either off chromatin or on promoters, is key for P-TEFb release from 7SK snRNP. Since phosphorylation of T186 is of key importance for P-TEFb's kinase activity, the re-phosphorylation of T186 is necessary for recovering P-TEFb's activity. However, how the dephosphorylated T186 of Cdk9 is re-phosphorylated again remains to be investigated. Collectively, although current studies

have identified different mechanisms for the recruitment of P-TEFb to promoters for pause release, there are still many unanswered questions. Future studies on this field should address the inducible recruitment and activation of P-TEFb.

#### **Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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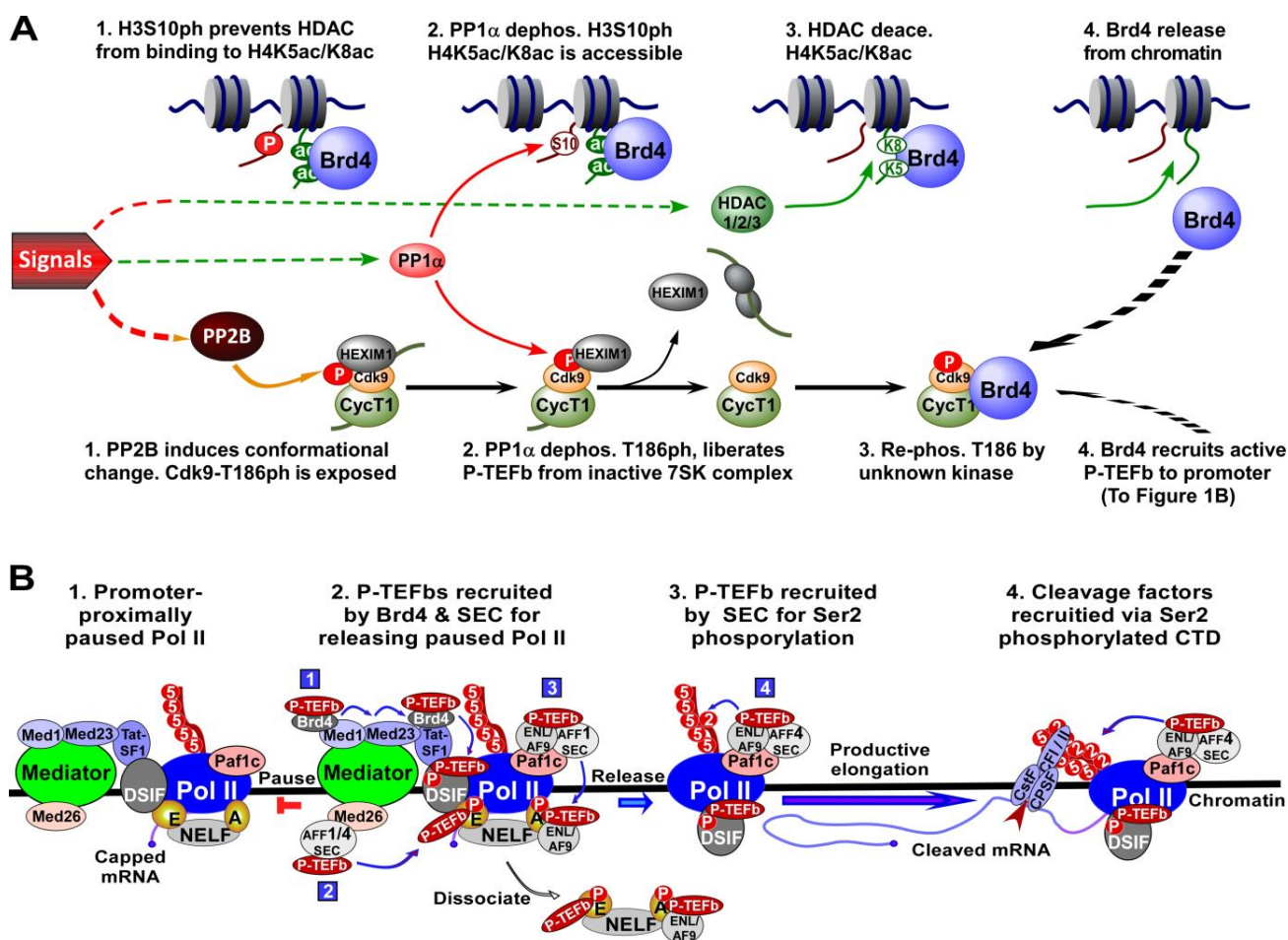
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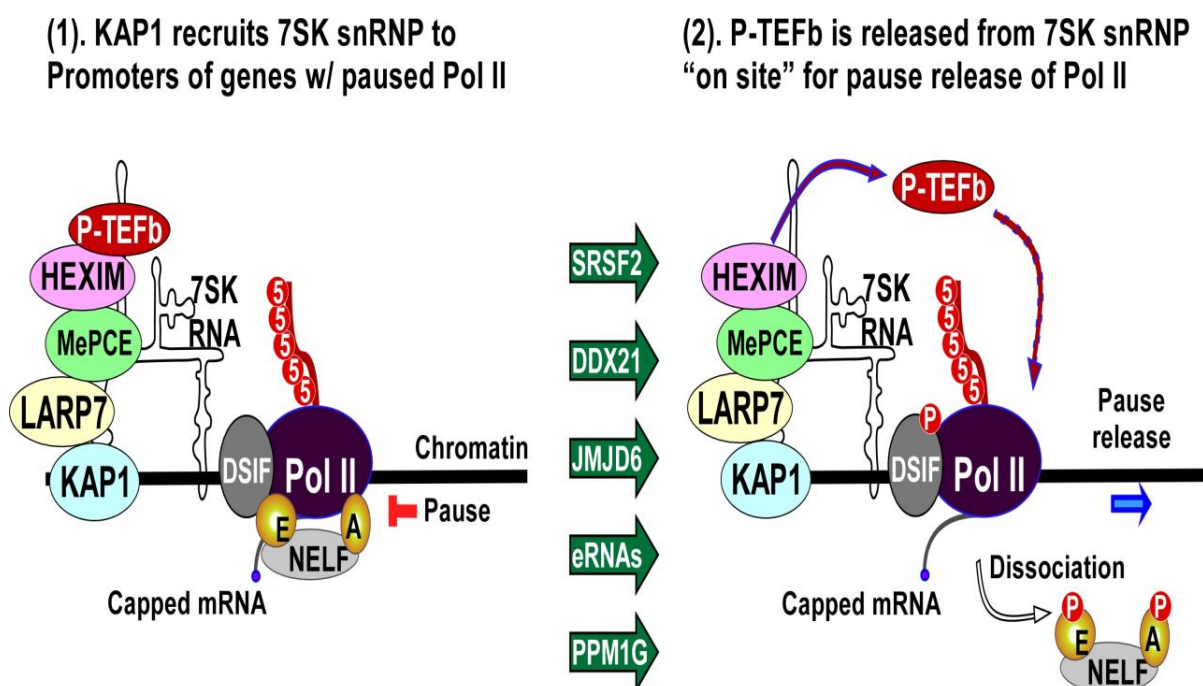
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**Figure 1. Canonical P-TEFb recruitment model.** **A.** PP1 $\alpha$  signal pathway coordinates the release of Brd4 and P-TEFb to form a recruitable P-TEFb/Brd4 complex. **(Top)** Stress-activated PP1 $\alpha$  signal pathway works together with class I histone deacetylases (HDAC1/2/3) for the release of chromatin-bound Brd4. Without stress, almost all Brd4 is locked up in nucleosomes containing H4K5ac/K8ac and H3S10ph. Upon stress, PP1 $\alpha$  dephosphorylates H3S10ph, allowing HDACs to access and deacetylate H4K5ac/K8ac to release nucleosome-bound Brd4. **(Bottom)** Stress-activated PP2B cooperates with PP1 $\alpha$  for release of P-TEFb from 7SK snRNP. The stress-activated Ca<sup>2+</sup>-dependent phosphatase PP2B induces conformational change of 7SK snRNP, allowing PP1 $\alpha$  to access and dephosphorylate T186ph of Cdk9 to release P-TEFb from 7SK snRNP. The released Brd4

binds to and recruits active P-TEFb to promoter region (see Figure 1B). **B.** Co-regulation of transcriptional pause release by multiple P-TEFbs which are recruited by Brd4 and SEC subtypes via a Mediator- and Paf1c-coordinated recruitment network. The number in black square denotes the number of P-TEFb recruited by either Brd4 or SEC as indicated.



**Figure 2. Summarization of non-canonical P-TEFb recruitment model.** First, 7SK snRNP is recruited by transcriptional regulators (such as KAP1) to promoter regions of genes with paused Pol II under either basal or stimulation state. Second, P-TEFb is released from 7SK snRNP "on site" by different factor as indicated. Finally, the "on site" released P-TEFb induces pause release by dissociation of NELF from paused Pol II.